

## WHAT IS CLAIMED IS:

1. A method for determining the presence or absence of a nucleic acid hybrid in a sample comprising the following steps:
  - (a) providing a reaction mixture comprising (i) a sample that may contain a nucleic acid hybrid that comprises a 3'-terminus, (ii) pyrophosphate, (iii) an enzyme that catalyzes the release of a nucleotide from a nucleic acid hybrid, by pyrophosphorolysis of the 3'-terminus of a strand of the nucleic acid hybrid in the presence of pyrophosphate, and (iv) a suitable nucleotide that can be incorporated in the place of said released nucleotide;
  - (b) maintaining said reaction mixture for a time period and under conditions that permit (i) pyrophosphorolysis of the 3'-terminus of a strand of a nucleic acid hybrid to produce a released nucleotide and a modified 3'-terminus as well as (ii) the incorporation of said suitable nucleotide into the modified 3'-terminus of the nucleic acid hybrid to produce an incorporated modified 3'-terminus, thereby forming a treated sample; and
  - (c) assaying the treated sample to determine whether incorporation of said suitable nucleotide into the hybrid occurred and thereby determining the presence or absence of a nucleic acid hybrid in said sample.
2. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 1 wherein the nucleotide suitable for incorporation into the modified 3'-terminus of the nucleic acid hybrid includes a label.
3. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 1 wherein the method of assaying whether incorporation of a suitable nucleotide occurred comprises determining whether the label is associated with the nucleic acid hybrid.
4. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 2 wherein the label is a fluorescent label.

5. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 3 wherein determining whether the fluorescent label is associated with the nucleic acid hybrid comprises the following steps:
  - (a) providing a treated sample comprising a fluorescence-modulating oligonucleotide that comprises a fluorescence quencher or enhancer that is complementary to the incorporated modified 3'-terminus having the fluorescent label;
  - (b) maintaining the treated sample under conditions and for a time period sufficient to permit hybridization of said incorporated modified 3'-terminus with said fluorescence-modulating oligonucleotide to form a fluorescence-modulated hybrid;
  - (c) irradiating the treated sample with radiation that is appropriate for causing fluorescence of said label; and
  - (d) monitoring the fluorescence of said treated sample comprising a fluorescence-modulating oligonucleotide, whereby a difference in fluorescence indicates the presence of a fluorescence-modulated hybrid and therefore said nucleic acid hybrid in the sample.
6. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 1 wherein said nucleic acid hybrid is formed between a target nucleic acid strand and a probe nucleic acid strand.
7. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 2 wherein the label is a capture label.
8. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 1 wherein said nucleic acid hybrid comprises a label.
9. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 8 wherein said label is a capture label.
10. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 2 wherein said nucleotide suitable for incorporation into the modified 3'-terminus of the nucleic acid hybrid is a chain terminating or other polymerization-blocking nucleotide.

11. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 6 wherein assaying to determine whether incorporation of said suitable nucleotide occurred is carried out by detecting an increase in the length of said probe.
12. A method for determining the presence or absence of a nucleic acid target in a sample comprising the following steps:
  - (a) providing a reaction mixture comprising (i) a sample that may contain a nucleic acid target, (ii) a nucleic acid probe corresponding to said nucleic acid target, (iii) pyrophosphate, (iv) an enzyme that catalyzes the release of a nucleotide from a nucleic acid hybrid, which comprises a 3'-terminus, by pyrophosphorolysis of the 3'-terminus of a strand of the nucleic acid hybrid in the presence of pyrophosphate, and (v) a suitable nucleotide that can be incorporated in the place of said released nucleotide;
  - (b) maintaining said reaction mixture for a time period and under conditions that permit (i) hybridization of the nucleic acid target with the nucleic acid probe to form a nucleic acid hybrid that comprises a 3'-terminus, (ii) pyrophosphorolysis of the 3'-terminus of a strand of a nucleic acid hybrid to produce a released nucleotide and a modified 3'-terminus as well as (iii) the incorporation of said suitable nucleotide into the modified 3'-terminus of the nucleic acid hybrid to produce an incorporated modified 3'-terminus, thereby forming a treated sample; and
  - (c) assaying the treated sample to determine whether incorporation of said suitable nucleotide occurred and thereby determining the presence or absence of the nucleic acid target in said sample.
13. The method for determining the presence or absence of a nucleic acid target in a sample according to claim 12 wherein said reaction mixture may comprise a plurality of nucleic acid targets and their corresponding nucleic acid probes.
14. The method for determining the presence or absence of a nucleic acid target in a sample according to claim 13 wherein said nucleic acid probes are distinguishable from one another on the basis of length, thereby permitting the determination of the presence or absence of a plurality of nucleic acid targets.

15. The method for determining the presence or absence of a nucleic acid target in a sample according to claim 13 wherein the suitable nucleotides incorporated to form the incorporated modified 3'-terminus permit distinction between the probes, thereby permitting the determination of the presence or absence of a plurality of nucleic acid targets.
16. The method for determining the presence or absence of a nucleic acid target in a sample according to claim 15 wherein the nucleic acid probes having incorporated modified 3'-termini are distinguishable from one another on the basis of the suitable nucleotide incorporated or on the basis of length, thereby permitting the determination of the presence or absence of a plurality of nucleic acid targets.
17. The method for determining the presence or absence of a nucleic acid target in a sample according to claim 12 wherein said reaction mixture may comprise a plurality of nucleic acid targets that differ from one another by a single base.
18. The method for determining the presence or absence of a nucleic acid target in a sample according to claim 17 wherein said plurality of nucleic acid targets differ from one another by a single base at an interrogation position.
19. The method for determining the presence or absence of a nucleic acid target in a sample according to claim 18 wherein the penultimate 3'-terminal residue of the corresponding nucleic acid probe base pairs with the interrogation position of the nucleic acid target.
20. A method for determining the presence or absence of a specific nucleic acid base at an interrogation position of a nucleic acid target in a sample comprising the following steps:
  - (a) providing a reaction mixture comprising (i) a sample that may contain a nucleic acid target having a nucleic acid residue at an interrogation position, (ii) a nucleic acid probe comprising a nucleic acid residue in its 3'-terminus that base pairs with the interrogation position of the nucleic acid target when the nucleic acid target and the nucleic acid probe are hybridized to form a nucleic acid hybrid, (ii)

pyrophosphate, (iii) an enzyme that catalyzes the release of a nucleotide from a nucleic acid hybrid, which comprises a 3'-terminus, by pyrophosphorolysis of the 3'-terminus of a strand of the nucleic acid hybrid in the presence of pyrophosphate, and (iv) a suitable nucleotide that can be incorporated in the place of said released nucleotide;

(b) maintaining said reaction mixture for a time period and under conditions that permit (i) formation of a nucleic acid hybrid between the nucleic acid probe and the nucleic acid target, (ii) pyrophosphorolysis of the 3'-terminus of a strand of a nucleic acid hybrid to produce a released nucleotide and a modified 3'-terminus and (iii) the incorporation of said suitable nucleotide into the modified 3'-terminus of the nucleic acid hybrid to produce an incorporated modified 3'-terminus, thereby forming a treated sample; and

(c) assaying the treated sample to determine whether incorporation of said suitable nucleotide occurred and thereby determining the presence or absence of a specific nucleic acid base at an interrogation position of a nucleic acid target in said sample.

21. The method for determining the presence or absence of a specific nucleic acid base at an interrogation position of a nucleic acid target in a sample according to claim 20 wherein the nucleic acid residue of the nucleic acid probe that corresponds with the interrogation position of the nucleic acid target is the 3'-terminal residue.
22. The method for determining the presence or absence of a specific nucleic acid base at an interrogation position of a nucleic acid target in a sample according to claim 20 wherein the nucleic acid residue of the nucleic acid probe that corresponds with the interrogation position of the nucleic acid target is the penultimate 3'-terminal residue.
23. A reaction mixture comprising (i) a sample that may contain a nucleic acid hybrid that comprises a 3'-terminus, (ii) pyrophosphate, (iii) an enzyme that catalyzes the release of a nucleotide from a nucleic acid hybrid, which comprises a 3'-terminus, by pyrophosphorolysis of the 3'-terminus of a strand of the nucleic acid hybrid in the presence of pyrophosphate, and (iv) a suitable nucleotide that can be incorporated in the place of said released nucleotide.

24. The method for determining the presence or absence of a nucleic acid hybrid in a sample according to claim 1 wherein said nucleic acid hybrid is affixed to a solid support.
25. The method for determining the presence or absence of a nucleic acid hybrid in a sample according to claim 24 wherein said nucleic acid hybrid is affixed to a solid support through attachment of a strand of the nucleic acid hybrid to said solid support.
26. The method of detecting the presence or absence of a nucleic acid hybrid according to claim 9 wherein said nucleic acid hybrid is attached to a solid support through said capture label and wherein said suitable nucleotide is a different label used for assaying the treated sample to determine whether incorporation of said suitable nucleotide occurred.
27. A method of determining a nucleotide sequence of a nucleic acid hybrid that comprises the following steps:
  - (a) providing a reaction mixture comprising (i) a sample contains a nucleic acid hybrid that comprises a 3'-terminus, (ii) pyrophosphate, (iii) an enzyme that catalyzes the release of a nucleotide from a nucleic acid hybrid, by pyrophosphorolysis of the 3'-terminus of a strand of the nucleic acid hybrid in the presence of pyrophosphate, and (iv) a suitable nucleotide that can be incorporated in the place of said released nucleotide;
  - (b) maintaining said reaction mixture for a time period and under conditions that permit (i) pyrophosphorolysis of the 3'-terminus of a strand of a nucleic acid hybrid to produce a released nucleotide and a modified 3'-terminus as well as (ii) the incorporation of said suitable nucleotide into the modified 3'-terminus of the nucleic acid hybrid to produce an incorporated modified 3'-terminus, thereby forming a treated sample; and
  - (c) assaying the treated sample to determine where incorporation of said suitable nucleotide into the hybrid occurred and thereby determining nucleotide sequence of the nucleic acid hybrid.

28. The method of determining a nucleotide sequence of a nucleic acid hybrid according to claim 27 wherein said nucleic acid hybrid is formed by a combination of a nucleic acid probe with a nucleic acid target.